UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

December 10, 2001

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 777-66/ Lysol Brand Direct Multi-Purpose Cleaner

DP Barcode: D276124
Case No.: 008461

From: Ian Blackwell, Biologist
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Regulatory Management Branch I
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Applicant: Reckitt Benckiser, Inc.

Formulation From Label:

Active Ingredient(s)	% by wt
Alkyl dimethyl benzyl ammonium chlorides	0.08
Alkyl benzyl ammonium chlorides	0.02
Inert Ingredient(s)	99.90
Total	100.00

I. **BACKGROUND:** Reckitt Benckiser, Inc., has submitted three antimicrobial efficacy studies to support the addition of sanitization labeling claims for their product, "Lysol Brand Disinfectant Direct Multi-Purpose Cleaner". The MRID Numbers are 454386-01 through 454386-03. MRID Number 454386-03 was conducted by Reckitt & Colman, Inc.'s Microbiology Laboratory. The other two studies were conducted by ViroMed Biosafety Laboratories, Inc.

II Use Directions

EPA Reg. No. 777-66 is packaged as a Ready-To-Use product in a trigger spray bottle. The label states: "**DISINFECT/DEODORIZE:** For heavily soiled surfaces, first clean according to General [Kitchen] Cleaning Directions. To disinfect/deodorize hard, nonporous surfaces such as floors, walls, counters, sinks, tubs and tile, apply full strength. Let stand 10 minutes before wiping."

The label also carries the following alternative language for sanitization claims:

- Kills [Eliminates] 99.9% of [bacteria] [viruses] on hard, non-porous surfaces in seconds.
- To sanitize/deodorize: Let stand for 30 seconds before wiping.
- Kills [eliminates] 99.9% of *Salmonella choleraesuis* (*Salmonella*), *Escherichia coli* (E. coli), *Staphylococcus aureus* (*Staphylococcus*), *Staphylococcus aureus* - MRSA, *Enterococcus faecalis* (VRE), *Enterobacter aerogenes*, Rhinovirus Type 39 and Rotavirus on hard, non-porous surfaces in 30 seconds.

III Agency Standards for Proposed Claims

As this product is a disinfectant/virucide/sanitizer, studies must be submitted that establish the efficacy of the product in these areas. As per the DIS/TSS -10, when testing products for their ability to sanitize non-food contact surfaces, "Three product samples, representing 3 different preparations, one of which is at least 60 days old, should be tested against each test bacterium on each test surface. The test bacteria are *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae*, aberrant, ATCC 4352. *Enterobacter aerogenes* (ATCC 13048 or 15038) may be substituted for *K. pneumoniae*. The results must show a bacterial reduction of at least 99.9% over the parallel control count within 5 minutes." Testing may be done to establish sanitization of surfaces contaminated with other organisms.

IV Comments on the Submitted Efficacy Studies

1. MRID Number 454386-01: "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Spray Product Application)" by Andrew T. Snyder. ViroMed Biosafety Laboratories. Project Number 7849. Study Completion Date 1/10/2000.

   This study was conducted to test the efficacy of "Formula Number V-2841-9" as a sanitizer of inanimate non-food contact surfaces contaminated or inoculated with *Enterococcus faecalis* - VRE (ATCC 51299). Five percent Fetal Bovine Serum was added to the bacterial culture as a soil load. Sterile carriers were inoculated with 0.03 mL of 48-54 hour cultures of the test bacteria. The product is sold as a pre-formulated pump spray, so no dilution or other preparation of the test substance was required. Each inoculated carrier was sprayed with 3 strokes of the test material holding the spray bottle at a distance of 6-8 inches from the carrier. Thirty seconds after spraying, each carrier was transferred to 20 mL of Letheen Broth to neutralize the sanitizer. Within 30 minutes after the addition of the neutralizer to the test solution, 1.0 and 0.1 mL of the neutralizer solution from each of the five jars was plated in duplicate using the standard spread plate technique and BAP. The plates were incubated at 35-37°C and plated for 48 hours prior to observation for number of colonies.

2. MRID Number 454386-02: "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Spray Product Application)" by Andrew T. Snyder. ViroMed Biosafety Laboratories. Project Number 7848. Study Completion Date 1/14/2000.

   This study was conducted to determine the ability of "Formula Number V-2841-9" to sanitize inanimate, non-food contact surfaces contaminated with Methicillin-Resistant *Staphylococcus aureus* (MRSA) (ATCC 33592). Five percent Fetal Bovine Serum was added to the bacterial culture as a soil load. Sterile carriers were inoculated with 0.03 mL of 49 hour cultures of the test bacteria. As the product is sold as a pre-formulated pump spray, no dilution or other preparation of the test substance was required. Each inoculated carrier was sprayed with 3 strokes of the test material holding the spray bottle at a distance of 6-8 inches from the carrier. Thirty seconds after spraying, each carrier was transferred to 20 mL of Letheen Broth to neutralize the sanitizer. Within 30 minutes after the addition of the neutralizer to the test solution, 1.0 and 0.1 mL of the neutralizer solution from each of the five jars was plated in duplicate using the standard spread plate technique and BAP. The plates were incubated at 36°C and plated for 48 hours prior to observation for number of colonies.

3. MRID Number 454386-03: "Disinfectant Efficacy Testing Non-Food Contact Type Sanitization Activity In The Presence of Organic Soil" by Kyle T. Smith. Reckitt & Colman, Inc. Microbiology Laboratory. Master Schedule Study Number 97.0094. Experimental Termination Date 5/12/97.

   This study was conducted to determine the ability of "Formula Number V-2841-9" to sanitize inanimate, non-food contact surfaces contaminated with *Salmonella choleraesuis* (ATCC 10708), *Escherichia coli* (ATCC 43888) or *Streptococcus pyogenes* (ATCC 12384). Five percent horse serum was added to the bacterial cultures as a soil load. A volume of 0.02 mL of test culture was placed onto 20 x 25 mm glass slides and allowed to dry. For each batch of the test substance, 2 slides were individually sprayed with 3 strokes of the test substance. The slides were left covered with the test substance for 30 seconds at 21.6-22.3°C. After the 30 second exposure, each slide was subcultured into 10 mL of Letheen Broth, vortexed, serially diluted, and plated onto Tryptic Soy Agar Plus Lecithin and Polysorbate 80. After incubation, the number of colonies per plate were counted and recorded.

V Results

MRID Number 454386-01 Non-Food Contact Sanitization		
Organism	Test Material Batch	Percent Reduction
Enterococcus faecalis - VRE (ATCC 51299)	629-017A	≥99.9%
Enterococcus faecalis - VRE (ATCC 51299)	629-017B	≥99.9%

MRID Number 454386-02 Non-Food Contact Sanitization		
Organism	Test Material Batch	Percent Reduction
Methicillin-Resistant *Staphylococcus aureus* (MRSA) (ATCC 33592)	629-017A	≥99.9%
Methicillin-Resistant *Staphylococcus aureus* (MRSA) (ATCC 33592)	629-017B	≥99.9%

MRID Number 454386-03 Non-Food Contact Sanitization		
Organism	Test Material Batch	Percent Reduction In Bacterial Count
Salmonella choleraesuis (ATCC 10708)	S6044A-8791-M01	100%
	S6059B	100%
	S6276-B	100%
Escherichia coli (ATCC 43888)	S6044A-8791-M01	100%
	S6059B	100%
	S6276-B	100%
Streptococcus pyogenes (ATCC 12384)	S6044A-8791-M01	≥99.9%
	S6059B	≥99.9%
	S6276-B	100%

VI Conclusions

1. MRID Number 454386-01: The submitted study support the use of "Formula Number V-2841-9" as a sanitizer of inanimate non-food contact surfaces contaminated with *Enterococcus faecalis* - VRE (ATCC 51299). However, the study is not currently acceptable for the following reasons:

   a. The test material is not identified as EPA Reg. No. 777-66.
   b. Although the study was conducted on *Enterococcus faecalis* - VRE (ATCC 51299), the report did not contain an antibiotic resistance study of the bacteria used in this study.

2. MRID Number 454386-02: The submitted study support the use of "Formula Number V-2841-9" as a sanitizer of inanimate non-food contact surfaces contaminated with Methicillin-Resistant *Staphylococcus aureus* (MRSA) (ATCC 33592).

   a. The test material is not identified as EPA Reg. No. 777-66.
   b. Although the study was conducted on *Enterococcus faecalis* - VRE (ATCC 51299), the report did not contain an antibiotic resistance study of the bacteria used in this study.

3. MRID Number 454386-03: This study supports the use of "Formula Number V-2841-9" as a sanitizer of inanimate non-food contact surfaces contaminated with *Salmonella choleraesuis* (ATCC 10708), *Escherichia coli* (ATCC 43888) or *Streptococcus pyogenes* (ATCC 12384). However, the study is not currently acceptable because the test material is not identified as EPA Reg. No. 777-66.

VII Recommendations

The requests to add labeling claims against the bacterial species used in these three studies is denied. In order to have the status of the three studies reconsidered, the registrant will have to do the following:

1. Properly identify "Formula Number V-2841-9" used in each of these three studies.
2. Submit antibiotic resistance studies on the bacteria used in MRID Numbers 454386-01 and 454386-02.